weeks after adoptive transfer, AML in bone marrow (BM) of PR1-CTL treated mice decreased 27% (range 21%-32%; $p < 0.02$) compared to untreated mice, and by 23% (range 4%-47%; p = NS) compared to mice receiving PDC. Moreover, AML in the PB of PR1-CTL treated mice decreased 47% (range 30%-61%, $p < 0.04$) compared to untreated mice, and by 58% (range 48%-68%; $p < 0.01$) compared to mice receiving PDC. These data justify clinical studies to determine whether PR1-CTL isolated directly from UCB can be used to enhance GVL without increased GVHD.

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ANTIGEN PRESENTING CELL-MEDIATED EX VIVO EXPANSION OF HUMAN UMBILICAL CORD BLOOD CELLS YIELDS SIGNIFICANT EXPANSION OF NATURAL KILLER CELLS WITH ANTI-MYELOMA ACTIVITY

Shah, N.[1], Ku, S.[2], Xing, D.[1], Lee, D.[3], Cooper, L.[3], Tzou, B.[2], Decker, W.[1], Li, S.[1], Robinson, S.[1], Yang, H.[1], Parmar, S.[1], Tung, S.[1], Bollard, C.[2], Shpall, E.J.[1] [1] M.D. Anderson Cancer Center, Houston, TX; [2] Baylor College of Medicine, Houston, TX; [3] M.D. Anderson Cancer Center, Houston, TX

Background: Allogeneic natural killer (NK) cells are active in various hematologic malignancies and may have an important role in multiple myeloma (MM). Umbilical cord blood (UCB) is a potential source for allogeneic NK cells and ex vivo expanded UCB-derived NK (UCB-NK) cells demonstrate activity comparable to that of peripheral blood-derived NK cells. Here we demonstrate the ability to expand fresh and frozen UCB-NK cells to clinical grade by novel techniques using artificial antigen presenting feeder cells modified to express IL-21 ("K562-cl9-mIL21") or IL-15 ("K562-mb-15-41BBL"). These UCB-NK cells demonstrate appropriate phenotype and are active against a variety of MM cell lines.

Methods: UCB-NK cells were expanded from 1) fresh or 2) frozen cord blood units. 1) Fresh cord blood mononuclear cells (CBMCs) were cultured in 10% human serum albumin media with IL-2 (500 IU/ml) and irradiated K562-cl9-mIL21 feeder cells (2:1 feeder: CBMC ratio) for 21 days. Thereafter, cells were subjected to CD3-immunomagnetic depletion. 2) Frozen CBMC's were grown in a gas permeable culture flask with IL-2 (100 IU/ml) and irradiated K562-mb-15-41BBL feeder cells (2:1 feeder: CBMC ratio). Cells were CD3-depleted on day 7, replated with the same conditions and grown until day 14. CD3-negative cells were then used as effector cells in functional assays. Flow cytometry was used to confirm NK cell purity (C56+/CD3- cells) and a standard chromium-51 assay was performed to determine NK cell cytotoxicity. Targets included K562 cells and MM cell lines RPMI 8226, ARP-1 and U266.

Results: Expansion of fresh and frozen CBMCs yielded a > 2000 and > 200 fold expansion of NK cells, respectively, compared with only 47 fold expansion of fresh CD56-selected cells cultured with IL-2 alone. After CD3 depletion, fresh and frozen-derived UCB-NK cultures were comprised of 92% and 94% CD56+/CD3- cells respectively. APC-expanded UCB-NK cells from fresh and frozen cords demonstrated cytotoxicity against the classic NK cell target K562 as well as MM cell lines RPMI 8226, ARP-1 and U266.

Conclusions: UCB-NK cells can be expanded ex vivo to clinically relevant doses for allogeneic NK cell therapy via co-culture with K562-cl9-mIL21 and K562-mb-15-41BBL feeder cells. Expanded UCB-NK cells are cytotoxic to K562 cells and various myeloma cell lines. Further study of fresh and frozen-derived UCB-NK cells as an adjunct therapy in stem cell transplantation for myeloma is warranted.

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TRANSFER OF SPECIFIC CELLULAR AND HUMORAL ANTI-TUMOR IMMUNITY AFTER STEM CELL TRANSPLANT (SCT) BY VACCINATING STAGE IV BREAST CANCER PATIENTS WITH Her2/*Neu* TARGETED T CELLS AND TRANSFERRING IMMUNE T CELLS INTO THE PATIENTS AFTER SCT

Thakur, A.[1], Al-Kadhimi, Z.[1], Paul, E.[1], Skuba, C.[2], Davol, P.[3], Steele, P.[1], Rathore, R.[3], Ayash, L.[1], Abidi, M.[1], Uberti, J.[1], Ratanatharathorn, V.[1], Lum, L.G.[1] [1] Karmanos Cancer Institute, Detroit, MI; [2] College Veterinary Medicine, East Lansing, MI; [3] Roger William Medical Center, Providence, RI

Women with stage IV metastatic breast cancer (BrCa) have limited treatment options. Innovative treatment strategies are needed to improve anti-tumor responses. We have recently shown that specific T cell immunity can be induced in patients by "vaccinating" the patients (pts) with infusions of anti-CD3 × anti-Her2/*neu* bispecific antibody (Her2Bi) armed activated T cells (aATC). This study investigated whether memory cytotoxic T lymphocytes (CTL) and antibody (Ab) directed at BrCa antigens can be transferred into patients after SCT by evaluating the cytotoxic and Ab responses after aATC infusions prior to SCT and after SCT. After infusions of aATC, peripheral blood lymphocytes (PBL) exhibited high levels of cytotoxicity directed at SK-BR-3 breast cancer cells and high serum levels of Th1 cytokines and IL-12. Three weeks after aATC infusions, ATC were expanded from the second leukopheresis and cryopreserved for re-infusion after SCT. The expanded ATC from 6 patients at an E:T ratio of 25:1 exhibited cytotoxicity ranging from 3.7-25.8 (mean13.6%) directed at the SK-BR-3. After SCT, pts received multiple infusions with a mean total of $54x10^9$ ATC (range 16-110 × 10^9). Cytotoxicity ranged from 4.7 to 70 % from 2 weeks to 12 months post SCT. We tested PBL for the transfer of humoral immunity after SCT by co-culturing PBL with or without SK-BR-3 tumor cells before IT, mid-IT, 1 month post-IT, pre and post SCT for *in vitro* specific anti-SK-BR-3 Ab synthesis. Anti-SK-BR-3 Abs detected in the culture supernatants by ELISA ranged from 15 to 40 ng/ml of Abs prior to IT, 150-220 ng/ml mid-IT and remained high between 70-95 ng/ml at 1 month post-IT. PBL after SCT showed gradual increases in *in vitro* Ab synthesis ranging from 0-10 ng at 1-month post SCT to 70-90 ng by six-month post SCT upon stimulation with tumor cells. Serum antitumor Ab levels directed at SK-BR-3 increased from 2-4 µg to 10-12 µg post IT and between 6-9 µg after SCT. No dose-limiting toxicities, delays in engraftment, and life-threatening infections were observed. These data show that transfer of pre-immunized ATC and T cells in the stem cell product enhance tumor specific cytotoxicity after HDC and PBSCT for breast cancer. Data suggest that both cellular and Ab responses can be transferred and provide an anti-tumor immune response early after SCT. This "vaccinate" and "boost" proof-of-principal strategy may be used to design protocols to enhance anti-tumor activity.

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IMPROVED POST-THAW STABILITY VALIDATION OF PERIPHERAL BLOOD CELL PRODUCTS UTILIZING THE INTRACELLULAR-LIKE CryoStor CRYOPRESERVATION SOLUTION, AND PRELIMINARY RESULTS OF CLINICAL APPLICATION

Fritsch, G.[1], Witt, V.[1], Matthes, S.[1], Dworzak, M.[1], Artwohl, M.[1], Clarke, D.[2], Mathew, A.J.[2] [1] St. Anna Kinderkrebsforschung, Vienna, Austria; [2] BioLife Solutions, Inc., Bothell, WA

Collection and cryopreservation of autologous stem cells is a routine procedure in a variety of malignant diseases. Further, improved stability of cell products is critical to the development of cell and tissue based therapies as part of the growth in regenerative medicine. A growing body of evidence indicates that one key method for improved cryopreservation efficacy is the utilization of a hyperosmotic/intracellular-like cryopreservation media, as opposed to the traditional use of an isotonic/extracellular-like media such as saline as the vehicle for the cryoprotectant. In this evaluation of cryopreservation methods for clinical application, cell samples from apheresis products were cryopreserved in a conventional isotonic-based freeze media (CFM; 20% DMSO, 10% human plasma derivate in Ringer solution) or intracellular-like CryoStor CS10 (CS10; 10% DMSO, serum-free and protein-free). Immediately after thawing, the recovery of WBC was 50.7 ± 14.4% for CFM versus 70 ± 11.6% for CS10 ($p < 0.001$), and that of CD34+ cells 81.8 ± 36.1% for CFM and 101 ± 16.4% for CS10 ($p < 0.05$). In CFM, 20 to 60 min after thawing there was a dramatic loss in cell viability (-40% to -90%), up to complete clotting in 3/10 samples. By contrast, cells remained viable up to 60 min after thawing in CS10, and no clotting occurred. Because of these positive validation results, cryopreservation in the intracellular-like CryoStor CS10 was translated to clinical application for treatment of hematological malignancies. To date, seven patients have received autologous stem cells cryopreserved in CS10.

Infusions were well tolerated, and both WBC > 1000 and ANC > 500 were reached on day +10.5 (7-11). CryoStor CS10 has demonstrated benefits in comparison to the previous standard CFM in terms of cell recovery post-thaw, particularly providing improved stability after 20 to 60 min storage at room temperature (RT) in these validation experiments, as well as demonstrating clinical tolerance in initial patient applications. Further data will be collected to validate the outcome of hematopoietic regeneration after reinfusion.

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INTRATHECAL AUTOLOGOUS TOTAL NUCLEATED CELLS FOR CHILDREN WITH HIPOXIC/ISCHEMIC BRAIN INJURY

Mancías-Guerra, M.C.[1], García-Rodríguez, F.[1], Marroquin-Escamilla, A.R.[2], González-Llano, O.[1], Rodríguez-Romo, L.N.[1], Barrera-Morales, D.C.[1], Elizondo-Riojas, G.[3], Garza-Alatorre, A.[2], Gómez-Almaguer, D.[1] [1]Hospital Universitario "Dr. José Eleuterio González", Monterrey, Nuevo León, Mexico; [2]Hospital Universitario "Dr. José Eleuterio González", Monterrey, Nuevo León, Mexico; [3]Hospital Universitario "Dr. José Eleuterio González", Monterrey, Nuevo León, Mexico

Background: Perinatal hypoxia/ischemia (H/I) is a brain disturbance due to the absence or decrease in oxygen supplementation in newborns, and represents a leading cause of neurologic injury. Neurological sequelae include cerebral palsy (CP), epilepsy, and cognitive deficit. The reparative, regenerative, and replicative properties of hematopoietic cells have shown that they could be a potential treatment of several neurological conditions, including chronic brain damage. It has been found that hematopoietic cells may be transported through the cerebrospinal fluid and be delivered more efficiently to the injured area after their introduction in the subarachnoid space of the spinal cord when compared with the intravenous route. The objective of this study is to report the safety and efficacy of the intrathecal autologous total nucleated cell injection in the treatment of children with H/I brain injury.

Methods: We included patients with H/I brain injury between 1 month and 8 years old. Patients were evaluated clinically with the Battelle Developmental Inventory (BDI) and with magnetic resonance images (MRI) before mobilization. Subcutaneous growth-colony stimulation factor was used for 4 days prior to the bone marrow (BM) harvest. The buffy coat was obtained through centrifugation of the BM. Total nucleated cells (TNC) were infused through lumbar puncture, and the red cell concentrate with a residual amount of TNC was administered intravenously.

Results: Eighteen patients were included, 13 male and 5 female with a median age of 3 (0 - 8) years and a mean weight of 12.6 (SD 6.7) kg. A median of 13.3 (1 - 39) $\times 10^6$ CD34+ cells in a volume of 7.5 (4 – 10.5) mL were infused intrathecally, and 5.45 (0.5 - 60) $\times 10^6$ CD34+ cells in 49.5 (6 – 181) mL were applied intravenously. Mild anesthetic complications were presented in 2 patients, while 4 had other minor side effects. Twelve patients have reached the 30-day follow-up, showing an increase in BDI equivalent age (11 [1 – 62] vs 11.5 [3 – 74] months, $P = .004$).Seven patients have completed the 6-month follow-up, increasing BDI equivalent age from 14 (1 - 62) to 17 (5 - 73) months ($P = .016$). There have been no changes yet in any of the MRI comparisons.

Conclusions: This procedure proved to be safe, with minimal side effects, and may result beneficial in patients' development. However, it will be necessary a phase II clinical trial to consider this approach as a therapeutic option.

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DELICATE BALANCE BETWEEN REGULATION AND STIMULATION AT THE ANTIGEN PRESENTING CELL SITE DETERMINES THE LIKELIHOOD OF SUCCESSFUL IN-VITRO PRIMING AND ENRICHMENT OF LEUKEMIA-REACTIVE T CELLS FROM THE NAÏVE DONOR REPERTOIRE

Jedema, I., Lam, T.S., van de Meent, M., Pots, J., Hoogstraten, C., Falkenburg, J.H.F. Leiden University Medical Center, Leiden, Netherlands

Although the in-vitro induction of leukemia-reactive T cells from the naïve donor repertoire has been shown to be feasible, the robustness of the procedure is limited. In this study, we investigated the role of the frequencies of antigen-specific precursor T cells (Tprec) and regulatory T cells (Treg), the number of antigen presenting cells (APC), and the number of targeted antigens (Ag) on the ability to prime, enrich and expand Ag-specific T cells from primary immune responses in-vitro. Therefore, we developed an in-vitro model system allowing quantitative and functional monitoring of Ag-specific activation and proliferation of individual donor T cells in the first 14 days of the immune response. In this model, we exposed naïve PKH-labeled Tprec to allogeneic APC in different responder/stimulator (R/S) ratios in the presence of different numbers of innocent bystander cells. Optimal T cell activation was seen at specific Tprec/S ratios between 1/1 and 1/5, irrespective of the number of innocent bystander cells. Lowering the number of stimulator cells per Tprec resulted in incomplete activation and proliferation, but more importantly, exposure to an excess of stimulator cells resulted in induction of activation-induced cell death (AICD) of the Ag-specific Tprec. Interestingly, when cells with an inferior APC phenotype (e.g. primary leukemic cells) were used as stimulator cells, activation curves were of similar shape but shifted towards higher APC amounts, resulting in optimal stimulation at 1/100 Tprec/S ratios. Treg were like Tprec attracted to the site of the APC and their activation further increased their inhibitory potential. Especially when they were at a numeric advantage, Treg were capable of impairing Ag-specific Tprec priming. Increasing the number of Ag-specific Tprec by simultaneous targeting of multiple antigens in combination with Treg depletion strongly increased the reproducibility of in-vitro priming and expansion of Ag-specific T cells. In conclusion, the in-vitro generation of Ag-specific primary immune responses can only be successfully and reproducibly performed by creating an optimal balance at the priming site of the immune response (e.g. the APC) between the numbers of negative regulators (Treg) and responding cells (Tprec). Using these insights we developed a standard operating procedure for the reproducible in-vitro induction and selection of leukemia-reactive T cells for adoptive transfer using leukemic APC as stimulator cells.

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CYTOTOXIC T CELLS SPECIFIC FOR ADENOVIRUS, BKV, CMV, EBV AND VZV PRODUCED FOR CLINICAL USE IN IMMUNE RECONSTITUTION POST ALLOGENEIC STEM CELL TRANSPLANTATION

Blyth, E.[1], Clancy, L.E.[2], Gaundar, S.[1], Simms, R.[1], Gottlieb, D.J.[1,2,3,4] [1]University of Sydney, Westmead, NSW, Australia; [2]Westmead Hospital, Westmead, NSW, Australia; [3]Westmead Hospital, Westmead, NSW, Australia; [4]University of Sydney, Westmead, NSW, Australia

Introduction: Adoptive immunotherapy using donor-derived cellular effectors can restore anti-viral immunity after allogeneic stem cell transplantation. We have developed a method for the production of a T cell product specific for multiple viruses that does not rely on EBV transformed cells for stimulation.

Table 1. Comparison of the BDI results between days 0 and +30.

Area	Personal/ Social	Adaptative	Motor skills	Comunication	Cognitive	Global	BDI equivalent age
Day 0	64.5 (3 - 154)	26.5 (4 - 97)	12.5 (3 - 157)	17.5 (5 - 115)	13 (1 - 93)	148 (28 - 576)	11 (1 - 62)
Day +30	67 (10 - 168)	28.5 (12 - 113)	19 (8 - 155)	19 (6 - 115)	14.5 (2 - 104)	156 (60 - 640)	11.5 (3 - 74)
P value*	.013	.054	.005	.005	.007	.004	.004

*Wilcoxon test